Research Proposal

On

'Method development & validation of some proton inhibitors, Anti psychotic and antiviral drugs By HPLC'

(Submitted to JK Lakshmipat University for Doctor of Philosophy in Chemistry)

Institute of Engineering and Technology
JK Lakshmipat University, Jaipur
2014

Supervisor

Dr.Mohd.Shahnawaz Khan
(Ph.D, FICC, MISCB)

Submitted by:

Mohit Jain
(Ph.D Scholar)

Introduction:

A key component of the quality of Active pharmaceutical drugs is the control of Impurities. It is important to identify and quantify the level of impurities that may be present to provide safe, effective and well controlled medicines. The identification and quantification of impurities to today's standards presents significant challenges to the analyst analytical chemist. The development of modern quantitative methods driven by these challenges and the rapid development of spectrometers has provided increasing opportunity to identify the structure & therefore the origin and safety potential, of such Impurities.

The pharmaceutical analytical chemistry is concerned with new analytical techniques and the analytical chemist should consider various principles related to interdisciplinary sciences such as chemistry, physics, biology, engineering, computer science, etc. in developing methods of analysis. For instance, the analytical instruments such as mass spectrometer developed by physicists found to have great applications in pharmaceutical analysis.

In the DMF (Drug Master File) holder or the ANDA (Abbreviated New Drug Application) applicant should be summaries those actual and potential Impurities most likely to arise during synthesis, purification and storage of the drug substance. This summary should be based on sound scientific appraisal of both chemical reactions involved in the synthesis and impurities associated with raw materials that could contribute to the impurities profile of the drug substance and also about possible degradation products. The studies (e.g. NMR, IR and MS) conducted to characterize the structure of actual Impurity or degradation product present in the drug substance at the apparent level of 0.1% or above (calculated using the response factor of drug substance) should be described. Hence, the bulk drug manufacturer should include authentic documented evidence for degradation products and impurities with the validated analytical method for quantification, along with structural elucidation reports before getting the registration or marketing approval. Analytical methods are required for a variety of reasons during drug development process. The regulatory agencies expect that any investigation of new drug or new drug product contains what is stated on the label

and in the correct amount over the shelf-life of the API or product. There are also further expectations about absence of any harmful contaminants and has not been otherwise adulterated. ICH (International Conference on Harmonization) [1] rules must ultimately be complied with regarding Impurities and degradation products. Pharmacopeia tests are also mandatory, even for investigational drugs and drug products.

Review of literature: It was found that some methods have been reported for these drugs individually or in combination with other drugs but no method has been reported so far for the above drugs. Some of the methods are presented below;

- **Pujeri and Khaderi**[2] have developed and validated a simple and sensitive RP-HPLC method for the determination of Omeprazole R-enantiomer (ROME) and omeprazole S-enantiomer (S-OME) in bulk drug samples and pharmaceutical formulations. The separation of R-OME and S-OME was achieved on a chiral AGP column using UV detector at 301 nm. The mobile phase consisted of 0.025 mol/ L di sodium hydrogen phosphate and Acetonitrile, (90:10, v/v) (pH 7.0)–. The linear range of detection was found to be 0.01–150 µg/ml (R2=0.9993) and 0.015–152 µg/ml (R2=0.9999) for R-OME and S-OME respectively. The method has been applied successfully for the determination of S-OME in pharmaceutical preparations. The excipients commonly presenting formulations did not interfere in the assay of S-OME.

- **Wankhede**[3] have developed and validated stability indicating simple, economic, rapid, precise and sensitive reverse phase high performance liquid chromatography method for determination of Omeprazole and Diclofenac sodium in capsule dosage form. It was performed on an BDS Hypersil C18, 250 mm X 4.6 mm, 5 µm, stainless steel analytical column from Thermoscientific with mobile phase consist of Ammonium acetate buffer (0.05M) :Acetonitrile (55:45 %v/v), pumped at a constant flow rate of 1 mL min-1 and UV detection at 289.0 nm. The method shows good peak shape, minimal tailing, with retention time 4.79 min and 6.62 min for Omeprazole and Diclofenac sodium. The both drugs was subjected to acidic, alkaline, oxidation, photo-degradation to apply stress

conditions. The developed method was able to separate degradation product generated under forced degradation studies. The developed method was validated as per ICH guidelines

- **Vyas**[4] have reported the development and complete validation of a stability indicating chiral high-performance liquid chromatography (HPLC) method for the enantioselective analysis of omeprazole in the enteric-coated formulations. A precise and sensitive enantiomeric separation of omeprazole was obtained on Chiralcel OD-H analytical column (250mm × 4.6 mm, 5µm particle size) using normal phase chromatography. The analysis was performed under UV detection at 301nm wavelength. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for (R)-omeprazole were found to be 0.39 and 0.78 µg/ml, respectively for 5 µl injection volumes. The percentage recovery of (R)-omeprazole ranged from 93.5 to 104 in spiked formulation samples and omeprazole sample solution and mobile phase were found to be stable for at least 24 h at room temperature. The proposed method was found to be suitable and accurate for the quantitative determination of undesired enantiomer in the enteric-coated omeprazole formulation.

- **Kirti** *et al*[5] have reported a simple, precise, specific and accurate normal phase HPLC method for the simultaneous determination of drotaverine hydrochloride and omeprazole in tablet dosage form. The chromatographic separation was achieved on HiQsil column using UV detector. The mobile phase consisting of n-heptane: dichloromethane: methanolic ammonia (5%): methanol at a flow rate of 1.0 ml/min was used. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

- **Santosh and samina**[6] have developed and validated a new, simple, high-performance thin-layer chromatographic method for determination of Rabeprazole sodium (RAB) and Domperidone (DOM) in combined tablet dosage form The mobile phase was toluene-acetone-methanol (4.5 + 4.5 + 0.5, v/v/v)

with UV detection at 285 nm. The method has been successfully applied for the analysis of drugs in a pharmaceutical formulation.

- **Maryam** *et al*[7] have developed a simple and rapid HPLC method for measuring of four proton-pump inhibitors (PPIs), Omeprazole (OPZ), Pantoprazol(PPZ), Lansoprazole(LPZ) and Rabeprazole(RPZ) concentrations in human plasma. A single step liquid–liquid extraction analytes along with an internal standard (IS) were separated using an isocratic mobile phase of phosphate buffer (10 mM)/acetonitrile (53/47, v/v adjusted pH to 7.3 with triethylamine) at flow rate of 1 mL/min on reverse phase TRACER EXCEL 120 ODS-A column at room temperature.

- **Harshal and Mukesh**[8] have reported a simple reversed- phase high performance liquid chromatography method for analysis of Omeprazole and its related substances in bulk material and commercial dosage forms. A gradient elution of filtered sample was performed on Zorbax XDB C8 (150 x 4.6), 5µ column with Glacine buffer (pH -8.8) as a mobile phase-A, Acetonitrile: Methanol (83:17) as a mobile phase-B , UV detection at 302 nm, at flow of 1.2 mL/min and maintaining the column temperature at 25°C.

- **Zarna Dedania** *et al*[9] have reported RP-HPLC method for simultaneous estimation of Omeprazole and Ondansetron in combined tablet dosage form. The mobile phase used was a combination of Methanol: Acetonitrile (90:10). The detection of the combined dosage form was carried out at 218 nm and a flow rate employed was 0.5 ml/min. The retention time for omeprazole and Ondansetron was found to be 5.39 and 11.08 min respectively. Linearity was obtained in the concentration range of 4 to 20 µg/ml of omeprazole and 4 to20 µg/ml of Ondansetron with a correlation coefficient of 0.997 and 0.9967.

- **Cristina and Marius**[10] have reported a gradient reversed phase liquid chromatographic (RP-LC) method and subsequently validated for the determination of Omeprazole and its process-related impurities. Separation was

achieved with a Zorbax extend C18 column and acetonitrile: water: triethylamine1% (pH adjusted to 9.5) as eluent, at a flow rate of 0.8 mL/min. UV detection was performed at 280 nm. The described method was linear over a range of 40.6- 203µg/mL for omeprazole, 0.9556-14.334 µg/mL for impurity A, 1.1568-17.352 µg/mL for impurity B, 1.0772-16.158µg/mL for impurity C, 1.289-19.344 µg/mL for impurity D and 0.7968- 11.952 µg/mL for impurity H. The accuracy of the method has been demonstrated at 5 concentration levels in the range of 60–140% of the specification limit and the recovery of impurities was found to be in the range of 90–109%. The method is simple, rapid, selective, accurate and useful for determining Omeprazole in dosage forms. The method can be useful in the quality control of bulk manufacturing and pharmaceutical formulations.

- **Kamrun** *et al*[11] have developed and validated a RP-HPLC method with UV detection has been validated to determine omeprazole concentrations in human serum and urine samples. The mobile phase consisted of a mixture of potassium dihydrogen phosphate buffer (pH 7.2 ± 0.05; 0.2 M) and Acetonitrile (70:30, v/v), pumped at a flow rate of 1.0 ml/min through the C-8 column at room temperature. Peaks were monitored by UV absorbance at 302 nm at a sensitivity of 0.0001 in concentrations ranging between 5 to 1000ng/ml for serum samples and 1 to 100µg/ml for urine samples. The recovery of omeprazole ranged from 95.68 to 99% and 95.54 to 99.8% for the serum and urine samples respectively. This method proved to be simple, accurate and precise for pharmacokinetic and bioequivalence studies of omeprazole.

- **Lakshmi and Anil**[12] have reported a simple reverse phase HPLC method for the determination of Omeprazole and Domperidone from tablet formulations. The determination was carried out on a Hypersil, ODS, C-18 (150×4.6 mm, 5 µ) column using a mobile phase of methanol:0.1 M ammonium acetate (pH 4.9) (60:40). The flow rate and runtime were 1 ml/min and 10 min, respectively. The eluent was monitored at 280 nm. The detector response was found to be linear in

the concentration range of 10-60 µg/ml for Omeprazole and 5-30 µg/ml for Domperidone.

- **Prasad and Steven**[13] have reported a sensitive and reliable HPLC method was validated for the simultaneous measurement of Omeprazole (OMP) and 5-hydroxyomeprazole (5OH-OMP) in human plasma. Analytes were resolved using a C-18 HPLC column and gradient elution mobile phase containing 50 mM phosphate buffer in acetonitrile (22 - 50% in 43 minutes followed by 15 minutes equilibration). The eluents were monitored by UV detection at 302 nm. The HPLC retention times were 12.19 min for 5OH-OMP, 30.25 min for OPC-18827 and 34.18 min for OMP with resolution factors of 28.3 for 5OH-OMP/ISD and 6.2 for ISD/OMP.

- **Mary et al**[14] have reported a stability-indicating HPLC assay method for the quantitation of omeprazole, to study the effect of pH on the stability of omeprazole and to quantify the drug in capsules. The excipients present in the capsules did not interfere with the assay procedure. The pH-rate profile curve indicated that the maximum stability was at pH 11. Below pH 7.8, the decomposition was very fast. The decomposition constants have a direct relationship with the H+ concentrations of the solutions.

- **Keyller and Antonia**[15] have reported a optimized method for the analysis of omeprazole (OMZ) by ultra-high speed LC with diode array detection using a monolithic Chromolith Fast Gradient RP 18 endcapped column (50 x 2.0 mm id). The analyses were performed at 30C using a mobile phase consisting of 0.15% (v/v) trifluoroacetic acid (TFA) in water (solvent A) and 0.15% (v/v) TFA in acetonitrile (solvent B) under a linear gradient of 5 to 90% B in 1 min at a flow rate of 1.0 mL/min and detection at 220 nm.

- **Nandini R. Pai, Deepnandan S. Dubhashi**[16] have reported a stability-indicating HPLC related substance method for the quantitation of aripiprazole, an isocratic reverse phase LC-method was developed using Phenomenex Luna C18, 150 x 4.6mm, 5µm column and a mobile phase comprising of Acetonitrile and

Phosphate buffer, 0.05M (40:60 v/v). The detector set at 227nm with flow rate of 1.0mL min

- **V. Vijaysree, D. Anantha Kumar,J.V.L.N. Seshagiri Rao**[17], have developed a Aripiprazole HPLC separation was carried out on a Symmetry C18 Xterra column (150 x 4.6mm; 5µ) using a mobile phase composed of methanol and potassium dihydrogen phosphate buffer (65:35 v/v), which was pumped at a flow rate of 0.6 mL/min. The drug in the eluate was monitored at 210 nm.

- **Bhadru Bhanotu, Srinath.P, Kedarnath.**[18] The proposed HPLC method is rapid ,sensitive, precise and accurate for the determination of Aripiprazole was chromatographed on a INERTSIL C18 column (250x4.6mm I.D., particle size 5 µm) in a mobile phase consisting of 0.02 M Sodium Dihydrogen Orthophosphate: Methanol in the ratio 30:70 v/v. The mobile phase was pumped at a flow rate of 0.8ml/min with detection at 283 nm.

- **A.S Jadhav,_D.B Pathare, M.S Shingare**[19] have developed chiral high performance liquid chromatographic method was developed and validated for the enantiomeric resolution of Valacyclovir.L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9h-purin-9-yl) methoxy] ethyl ester, an antiviral agent in bulk drug substance. The enantiomers of Valacyclovir were resolved on a Chiralpak AD (250mm×4.6mm, 10µm) column using a mobile phase system containing*n*-hexane: ethanol: diethylamine (30:70:0.1, v/v/v). The resolution between the enantiomers was found not less than four. The presence of diethylamine in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (D)-enantiomer were found to be 300 and 900 ng/ml, respectively, for 20 µL injection volume.

- **G. Raveendra Babu, J. Srinivasa Rao, K. Suresh kumar and P. Jayachandra Reddy**[20] have developed stability indicating liquid chromatographic assay method

was developed and validated as per ICH guide lines for the quantitative estimation of Aripiprazole in tablet formulation. An isocratic reverse phase LC-method was developed using Phenomenex Luna C18, 150 x 4.6mm, 5µm column and a mobile phase comprising of Acetonitrile and Phosphate buffer, 0.05M (40:60 v/v). The detector set at 227nm with flow rate of 1.0mL min-1.

- **Yasmeen Sultana, Nanda Kishore Agarwal, Safia Khanam.P**[22] developed rapid RP-HPLC method for determination of Valacyclovir in bulk and pharmaceutical dosage forms. Valacyclovir was found to be degraded under different set of conditions as followed according to ICH guidelines and the degradants so formed along with Valacyclovir are separated by using Hypersil BDS C18 150mm×4.6mm×5µm using mobile phase comprising of mobile phase-A (sodium dihydrogen phosphate monohydrate buffer PH 3.51 with Orthophosphoric Acid) and mobile phase-B (acetonitrile: methanol, 60:40), with a flow rate of 1.5ml/min with a detection wavelength of 254nm with a injection volume of 10µl and the method was validated for specificity, linearity, accuracy, robustness and precision.

- **Arianna Loregian, Rosalba Gatti, Giorgio Palù, Elio F De Palo**[23] Acyclovir (ACV), Valaciclovir (VACV), and other analogues such as ganciclovir and penciclovir are developed here. The reversed-phase (RP) HPLC is widely used for the analysis. C_{18}Silica columns from 7.5 to 30 cm in length are used, the separation is carried out mainly at room temperature and less than 10 min is sufficient for the analysis at 1.0–1.5 ml/min of flow-rate. The separation methods require an isocratic system, and various authors have proposed a variety of mobile phases. The detection requires absorbance or fluorescence measurements carried out at 250–254 nm and at λ_{ex}=260–285 nm, λ_{em}=375–380 nm, respectively. The detection limit is about 0.3–10 ng/ml but the most important aspect is related to the sample treatment, mainly when body fluids are under examination. The plasma samples obtained from human blood are pre-treated with an acid or acetonitrile deproteinization and the supernatant after centrifugation is successively extracted before RP-HPLC injection.

Objectives:

The objective of the present study is to develop and validate a accurate, precise and rapid RP-HPLC method for estimation of proton inhibitors, Anti psychotic and antiviral drugs.

- Step 1- Selection of the RP-HPLC method and initial system.
- Step 2- Selection of initial conditions.
- Step 3- Selectivity optimization.
- Step 4- System optimization.

Method development involves a series of sample steps; based on what is known about the sample, a column and detector are chosen; the sample is dissolved, extracted, purified and filtered as required; an eluent survey (isocratic or gradient) is run; the type of final separation (isocratic or gradient) is determined from the survey; preliminary conditions are determined for the final separation; retention efficiency and selectivity are optimized as required for the purpose of the separation (quantitative, qualitative or preparation); the method is validated using ICH guidelines. The validated method and data can then be documented.

Validation is necessary for the following reasons:

1. To determine whether the process is under control.
2. To setup the appropriate in-process control.

Typical analytical parameters according to ICH Q2 (R1)[1] guideline can be used in assay and related substance validation include:

1. Specificity
2. Quantitative limit and Detection limit
3. Linearity
4. Accuracy
5. Precision
6. Repeatability
7. Intermediate Precision
8. Reproducibility
9. Range & Robustness

Proposed methodology during research work:

- Method shall be developed using HPLC (Waters), electronic balance (Sartorius), UV detector in the following steps:
- Selection of mobile phase based on trial and error method to get good resolution and sharp peaks for the drug.
- Dosage formulation shall be procured from local market.
- Pure sample of Rabeprazole Sodium, Omeprazole, Aripiprazole, Esomeprazole magnesium and Valacyclovir Hydrochloride shall be procured from industries involved in bulk manufacturing of these drugs.
- Determination of optimum wavelength and optimum flow rate.
- Standardization of retention time.
- The developed method shall be validated by using various validation parameters like accuracy, precision, specificity, robustness, ruggedness, linearity and range.
- To study the effect of forced degradation on drugs (in acidic and basic conditions, on oxidation and on increased temperature) and to determine the degraded products.
- To study the effect of related substances on drugs.
- The developed and validated method shall be applied for determination of drugs and its related substances in formulation.

Scope of proposed research work :

Scope of this study includes providing a comprehensive analytical method development studies for isolation, identification and quantification of impurities in drug substances and intermediates, covers the following topics.

A- Identification, isolation and characterisation of impurities of drug substances.

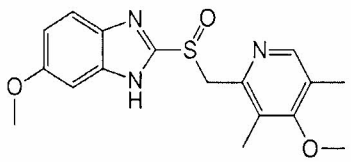
B- Development and validation of analytical methods for advanced drug intermediates.

C- Development of stability indicating LC methods and validation of analytical methods for Active Pharmaceutical Ingredients such as proton pump inhibitors (benzimadazole derivatives such as Rabeprazole Sodium) and anti psychotic drugs (quinoline derivatives such as Aripiprazole).

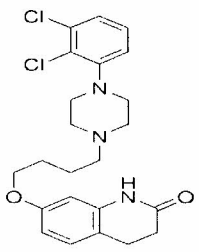
D- Analytical method development by chromatography (HPLC, GC...) and validation of Stability Indicating Assay Methods (SIAM) and Stability Indicating Related substance Methods (SIRSM) for qualification of developed analytical methods. Sometimes the impurities can be enriched by forced degradation studies which intern the part of the analytical method validations. The validation of analytical procedures in compliance with international regulatory guidelines assures the quality of the product for regulatory purpose and can be easily marketed in highly regulated markets like US, Europe and Japan.

The proposed project work aims the quantification of related substances with more accuracy and precision of the following drugs:-

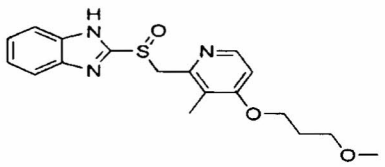
- Omeprazole



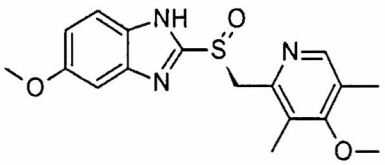
- Aripiprazole



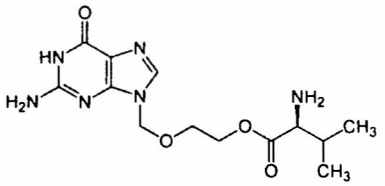
- Rabeprazole sodium



- Esomeprazole magnesium



- Valacyclovir hydrochloride



Noteworthy contributions in the field of proposed work:

Drug analysis play very Important role in creating the basis for high efficient drug therapy by giving analytical support to synthetic, biotechnological, pharmacological, pharmaceutical technological, clinical research to find the most efficacious drug material and its optimal dosage form. Impurity profiling can be defined as a group of analytical activities aiming at identification/structural elucidation and quantitative determination of organic and inorganic Impurities as well as residual solvents in bulk drugs and pharmaceutical formulations.

The purpose behind stability testing of a drug is that, to monitor quality of a drug product which may vary with time because of the influence of many of the environmental conditions such as temperature, light, humidity etc. The results are applied in developing manufacturing processes and selecting proper packaging, storage conditions, product's shelf life and expiry dates.

Stress testing of the drug can help to identify the degradation products which can help to establish degradation pathways and the intrinsic stability of the molecule and validate stability indicating power for analytical procedures used. Validation of a method indicates to establish documented evidence that the system is doing what is purpose to do. Validation is necessary when a method or a procedure is going to be used by a manufacturing company or to be published in any Pharmacopoeias. The validated assay methods will be more accurate, precise and reproducible. Hence, the bulk drug manufacturer should include documented evidence for degradation products and Impurities with the validated analytical method for quantification, along with structural elucidation reports before getting the registration or marketing approval. This subject or topic for the research activity is selected based on the increasing needs of the pharmaceutical industry in developing suitable analytical methods. Among the various other available techniques, the scope of work was focused on the modern chromatographic techniques such as HPLC which are accurate, precise and sophisticated techniques and are having wide spectrum of application in pharmaceutical industry. Literatures were reviewed and it was felt that there is a need to develop new, simple, specific, reliable analytical methods for Rabeprazole Sodium, Omeprazole, Aripiprazole, Esomeprazole magnesium and Val acyclovir Hydrochloride drug.

References:

1. Validation of Analytical Procedures: Text and Methodology. (Q2R1), ICH Harmonised Tripartite Guideline.

2. Pujeril SS, Khaderi AMA and Seetharamappa J. Development and Validation of LC method for the assay of Omeprazole enantiomers in pharmaceutical formulations .Der pharmacia Letter. 2012; 4(1): 76-86.

3. Wankhede S B. Stability indicating determination of Omeprazole and Diclofenac sodium in pharmaceutical preparation by RP-HPLC.http://www.inventi.in/Article/ppaqa/231/11.aspx.

4. Vyas S, Patel A, Ladva KD. Development and validation of a stability indicating method for the enantioselective estimation of omeprazole enantiomers in the enteric coated formulations by HPLC. J Pharm Bioallied Sci. 2011: 3(2): 310-14.

5. Kirti ST, Rajesh MJ, Purushotam KS and Mrinalini C D. A Validated normal phase HPLC method for simultaneous determination of Drotaverine hydrochloride and Omeprazole in Pharmaceutical formulation. Asi J Pharm and Clin Res. 2010; 3(1): 20-4.

6. Santosh VG, Samina IK. Stability indicating high performance thin layer chromatographic method for determination of Omeprazole in capsule dosage form. Journal of AOAC Inter. 2010; 93(3): 787-91.

7. Maryam N, Fariborz K ,Manijeh M. Improved HPLC Method for determination of four PPI,s Omeprazole,Pantaprazole, Lansoprazole,and Rabeprazole in human plasma. J Pharm Pharm Sci 2010; 13(1): 1-10.

8. Harshal KT, Mukesh CP, Development and validation of a precise single HPLC method for determination of omeprazole and its related compound in pharmaceutical formulation. Int J of Chem Tech Res. 2010; 2(3): 1355-67.

9. Zarna D, Ronak D, Vaishali K. RP-HPLC Method for Simultaneous Estimation of Omeprazole and Ondansetron in combined Dosage Forms. Asian J Res Chem. 2009; 2(2): 108-111.

10. Cristina I, Marius BSL .Development of a validated RP HPLC method for separation and determination of process related impurities of omeprazole in bulk drugs. FARMACIA. 2009; 57(5): 534-41.

11. Kamrun N, Jafreen JJ, Md Ashik Ullah. Simple RP-HPLC method for the determination of omeprazole in human serum and urine: Validation and application in pharmacokinetic study. Dhaka Univ J Pharm Sci. 2009; 8(2): 123-30.

12. Lakshmi S, Anil KV. Simultaneous HPLC estimation of omeprazole and domperidone from tablets. Ind J ph Sci. 2007; 69 (5): 674-76.

13. Prasad NV, Tata SLB High Performance Liquid chromatographic method for the analysis of Omeprazole and 5 –hydroxy omeprazole in human plasma. Analytical letters. 1999; 32(11): 2285-95.

14. Mary M, Das GV and Rodney EB. Stability of omeprazole solutions at various pH values as determined by HPLC. Drug Development and Industrial Pharmacy. 1995; 21(8): 965-71.

15. Keyller BB, Antonio JMS. Ultra-fast gradient LC method for Omeprazole analysis using a monolithic column. J AOAC Inter; 93(6): 1811-20

16. Nandini R. Pai *et al* stability-indicating HPLC related substance method for the quantitation of aripiprazole Der Pharmacia Lettre, 2010, 2(4): 1-10

17. Vasanthraju et al development & valdation of stability indicating HPLC method for determination of solifenacin in bulk formulations. Int J Pharm Pharm Sci, Vol 3, Issue1, 7074

18. Bhadru Bhanotu et al Development, Estimation and Validation of Aripiprazole in Bulk and Its Pharmaceutical Formulation by HPLC Method. Int.J. ChemTech Res.2012,4(1)

19. A.S Jadhav,D.B Pathare,M.S Shingare developed chiral high performance liquid chromatographic method was developed and validated for the enantiomeric resolution of Valacyclovir, L-valine2-[(2-amino-1,6-dihydro-6-oxo-9h-purin-9-yl) methoxy] ethyl ester, an antiviral agent in bulk drug substance. Journal of Pharmaceutical and Biomedical Analysis Volume 43, Issue 4, 12 March 2007, Pages 1568–1572

20. G. Raveendra Babu, J. Srinivasa Rao, K. Suresh kumar and P. Jayachandra Reddy developed a Stability Indicating Liquid Chromatographic Method for Aripiprazole, Asian J. Pharm. Ana., 2011,1, 03-07.

21. Bhadru Bhanotu, Srinath.P, Kedarnath.J, Developmed Estimation and Validation of Aripiprazole in Bulk and Its Pharmaceutical Formulation by HPLC Method, IJCRGG Vol.4, No.1, pp 124-128, Jan-Mar 2012

22. Yasmeen Sultana, Nanda Kishore Agarwal, Safia Khanam.P Developed and Validation of Stability Indicating RP-HPLC Method for Estimation of Valacyclovir in Pharmaceutical Dosage Forms, IJPCR, January-March 2013, Vol 5, Issue 1, 7-12

23. Arianna Loregian, Rosalba Gatti, Giorgio Palù, Elio F De Palo, Developed the Separation methods for acyclovir and related antiviral compounds. Journal of Chromatography B: Biomedical Sciences and Applications Volume 764, Issues 1–2, 25 November 2001, Pages 289–311